Click Chemistry Labeling of Oligonucleotides and DNA

Click chemistry is a versatile reaction that can be used for the synthesis of a variety of conjugates. Virtually any biomolecule can be easily labelled with small molecules, such as fluorescent dyes, biotin, etc using click chemistry method.

Click chemistry reaction takes place between two components: **azide** and **alkyne (terminal acetylene)**. Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific. If there is a need to label an **oligonucleotide**, alkyne-modified oligonucleotides can be ordered at many of the custom oligo-synthesizing facilities and companies.

Protocol

We recommend using the following general protocol for click chemistry labeling of alkyne-modified oligonucleotides with azides produced by Lumiprobe Corp. The auxiliary reagents can be ordered at Lumiprobe Corp.

1. Calculate the volumes of reagents required for Click chemistry labeling using the table below. Prepare the required stock solutions (see Appendix).

Reagent	Final concentration in the mixture	Stock solution concentration
Oligonucleotide, alkyne-modified	Varies (20 — 200 uM)	Varies
Azide	1.5 x (oligonucleotide concentration)	10 mM in DMSO
DMSO	50 vol %	—
Ascorbic acid	0.5 mM	5 mM in water
Cu-TBTA complex	0.5 mM	10 mM in 55 vol % DMSO

2. Dissolve **alkyne-modified oligonucleotide** or DNA in water in a pressure-tight vial.
3. Add **2M triethylammonium acetate buffer, pH 7.0**, to final concentration 0.2 M.
4. Add **DMSO**, and vortex.
5. Add **azide stock solution** (10 mM in DMSO), and vortex.
6. Add the required volume of **5mM Ascorbic Acid Stock solution** to the mixture, and vortex briefly.
7. Degass the solution by bubbling inert gas in it for 30 seconds. Nitrogen, argon, or helium can be used.
8. Add the required amount of **10 mM Copper (II)-TBTA Stock in 55% DMSO** to the mixture. Flush the vial with inert gas and close the cap.
9. Vortex the mixture thoroughly. If significant precipitation of azide is observed, heat the vial for 3 minutes at 80°C, and vortex.
10. Keep at room temperature overnight.
11. Precipitate the conjugate with acetone (for oligonucleotides) or with ethanol (for DNA).

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To precipitate an oligonucleotide conjugate:

add to the mixture at least a 4-fold excess volume of 3% lithium perchlorate in acetone (if the volume of the mixture is large, split in several vials).

To precipitate a DNA conjugate:

add to the mixture sodium acetate to a final concentration of 0.3 M;

add 2.5 volumes of ethanol (or 0.8 volumes of isopropanol).

Mix thoroughly and keep at -20 °C for 20 minutes.

12. Centrifuge at 10000 rpm for 10 minutes.
13. Discard the supernatant.
14. Wash the pellet with acetone (1 mL), centrifuge at 10000 rpm for 10 minutes.
15. Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.

Appendix. Preparation of stock solutions of the reagents used for click-chemistry labeling and conjugation

5 mM Ascorbic Acid Stock

Preparation Dissolve 18 mg of ascorbic acid in 20 mL of distilled water.

Storage Ascorbic acid is readily oxidized by air. The solution is stable for one day. Use fresh preparations for Click chemistry.

10 mM Copper (II)-TBTA Stock in 55% DMSO

Preparation Dissolve 50 mg of copper (II) sulfate pentahydrate in 10 mL of distilled water. Dissolve 116 mg of TBTA ligand in 11 mL of DMSO. Mix two solutions.

Storage Store at room temperature. The solution is stable for years.

2M Triethylammonium Acetate Buffer, pH 7.0

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Preparation

Mix 2.78 mL of triethylamine with 1.14 mL of acetic acid. Add water to 10 mL volume, and adjust pH to 7.0.

Storage

Store at room temperature. The solution is stable for years.

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